Adaptations for Carbon and Energy Metabolism in *Psychrobacter* 273-4

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Psychrobacter 273-4 was isolated after 10,000-40,000 yr exposure to continuous subzero temperatures and low water activity. Understanding the genetic and physiological adaptations that Psychrobacter has made to inhabit permafrost will enable us to hypothesize about potential microbial life in extraterrestrial cryo environments. In this study, we asked the question, "What carbon sources has *Psychrobacter* adapted to use for optimal growth at low temperatures?" Metabolic pathway analysis indicated that, despite previous expectations, *Psychrobacter* was not adapted to grow on sugars, but appeared to have the capacity to grow on carboxylic acids. Metabolism of the 2-C compound glyoxylate is likely accomplished through an unusual pathway ending in 3-hydroxy-aspartate. Microarray comparisons of *Psychrobacter* growth at 22ºC vs 4ºC showed that *Psychrobacter* upregulates genes for glycolate uptake and glyoxylate metabolism at 4ºC suggesting increased demand for carbon and energy from this substrate during low temperature metabolism. Sequence analysis of *icl* (isocitrate lyase) gene has shown that it clusters with *icl* genes from other cold adapted organisms. These data led to the hypothesis that *Psychrobacter* is adapted for metabolism of carboxylic acids, particularly the 2-C compounds acetate and glyoxylate. *Psychrobacter* was grown in a defined media containing several different compounds. Lactate, pyruvate, and acetate all supported growth as sole carbon sources. Growth rates on acetate were more than thrice the rates

observed in 1/2 tryptic soy broth at 4ºC. This work shows *Psychrobacter* is adapted to use oxidized substrates, and suggests the glyoxylate bypass (involving *icl*) plays an important role in low temperature energy metabolism.